OXYGEN UPTAKE IN SHORT PIECES OF TUBULARIA STEMS

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Short pieces (1–1½ mm. long) of *Tubularia* stems reconstitute miniature hydranths or partial hydranths. Unusually long periods are required for reconstitution of these pieces, and hydrogen ion estimates with indicators show low pH of the coelenteric fluid of such pieces (Miller and Miller, unpublished data). Since increased O_2 in the surrounding sea water increases the number of pieces which reconstitute and the size of the organ primordia, it became of significance to determine whether or not the oxygen uptake of 1- and 1½-mm. pieces was depressed as compared with that of longer stem segments.

In addition, the previously published studies on oxygen uptake in *Tubularia* (Hyman, 1926; Barth, 1940b; Sze, 1953) have resulted in a certain degree of confusion regarding the role of oxygen in reconstitution. On the one hand, whether or not a hydranth develops and the size of the hydranth which reconstitutes at a cut surface depend upon the oxygen available to the stem (Barth, 1938; Miller, 1937, 1942; Zwilling, 1939); on the other hand, oxygen uptake measurements during reconstitution failed to show that reconstituting stems used an appreciable amount of oxygen more than stems with both ends ligatured (Barth, 1940b). Since reconstitution in *Tubularia* involves cell migrations (Bickford, 1894; Steinberg, 1955), dedifferentiation and redifferentiation (Bickford, 1894), all processes which require energy, logic would demand an appreciable increase in O_2 uptake during these activities. Likewise, there were certain technical problems in both Barth's and Sze's methods which made it desirable for their results to be checked in another laboratory. Therefore, a new study of oxygen requirements in *Tubularia* was initiated in the summer of 1961 using the cartesian diver equipment of the Single Cell Research Foundation.[1]

Materials and Methods

Specimens of *Tubularia crocea* were collected in the region of Woods Hole, Mass., and were maintained in aerated running sea water in the laboratory. During the first half of the summer they came from the warmer waters of the south side of Cape Cod. During the last half they were collected from the Cape Cod Canal where the temperature seldom rises above 15° C.

Straight stems of uniform thickness were selected from a single bunch of the stock supply and placed in filtered sea water to which had been added 16×10^{-5} gm./ml. streptomycin to provide bacteriostasis. The stems remained in this solution in a cold room (18° C.) for at least 12 hours. The hydranth plus 5 mm. of the stem were then removed and the required length of stem was cut from the

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region immediately proximal. Since the accuracy of the results depended upon the accuracy of the measurements of length and diameter of the stem segments, the sizes of the pieces were checked under a microscope with an ocular micrometer, and stems which varied were discarded.

Oxygen uptake measurements were made in the Claff modification of the Holter (1943) and Linderstrøm-Lang (1943) cartesian diver apparatus with 7 flotation tubes suspended in a water bath maintained at 18° C. ±.01° C. For each series of measurements six of the tubes contained divers with tubularian stems and the seventh contained an unfilled diver to act as a check on the equipment. A "braking" pipette (Claff, 1947) was used to insert stem pieces plus 2 mm.^{3} of incubating solution into the divers. The necks of the divers were sealed with 2 mm.^{3} NaOH to absorb CO_2 and 2 mm.^{3} paraffin oil to prevent diffusion of gases. After sufficient time for the divers to equilibrate, manometer readings were taken every 10 minutes for a minimum of two hours.

Results

Since oxygen uptake data obtained on single 3-mm. stems were used as a standard of reference, in each set of measurements at least one diver contained a 3-mm. stem, the "control." In the various experiments to be described the oxygen up-

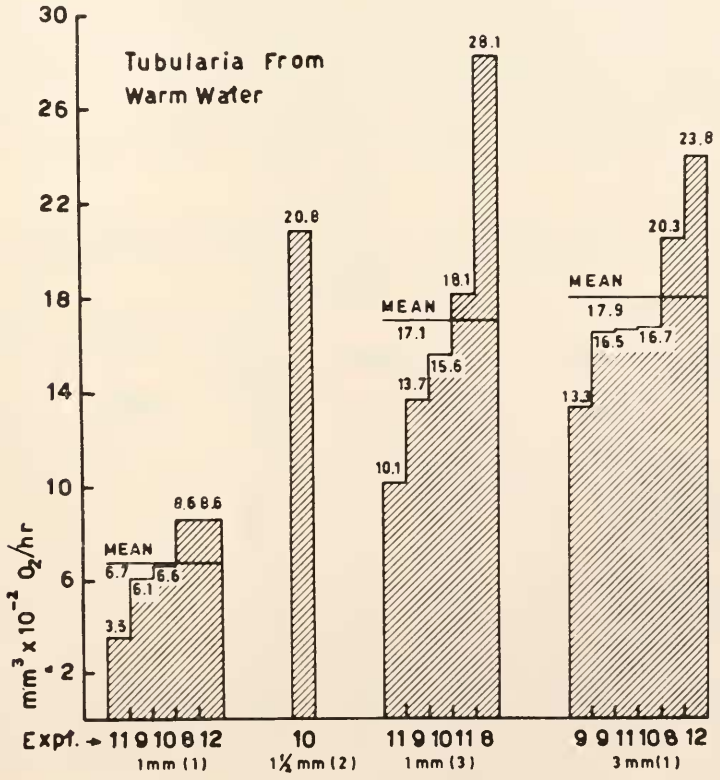

Figure 1.

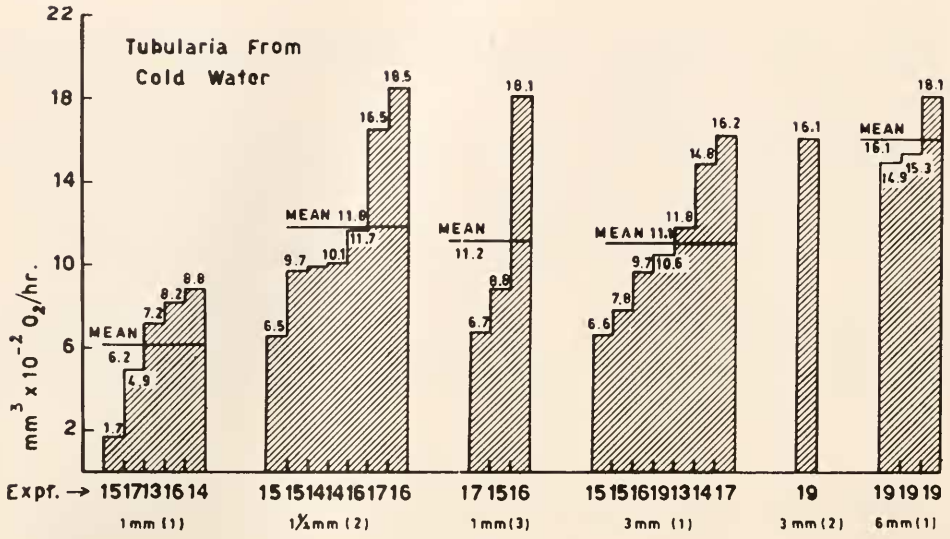

FIGURE 2.

takes of the following were compared with that of a single 3-mm. stem: (1) a single 1-mm. piece, (2) three 1-mm. pieces, (3) two 1½-mm. pieces, (4) two 3-mm. pieces and (4) one 6-mm. piece (Figs. 1, 2).

Table I summarizes the O_2 uptake measurements on 43 stems of various lengths from 1 mm. to 6 mm. and measured either singly (Columns 1, 4, 6), in pairs

TABLE I

O_2 UPTAKE OF 1, 1½, 3 AND 6 MM STEMS OF TUBULARIA

Source of Stems	Total Stem Length 3 mm			Total 1 mm	Total Length 6 mm	
	3 mm	1½ mm	1 mm	1 mm	3 mm	6 mm
Warm Water (WOODS HOLE)	17.9	20.8*	17.1	6.7		
Cold Water (COD CANAL)	11.1	11.8	11.2	6.2	16.1*	16.1
MEANS	14.2	13.0	14.9	6.4	16.1*	16.1
Column	1	2	3	4	5	6

* Tentative: More data needed

(Columns 2, 5), or in threes (Column 3). As may be seen in the averages little difference was found between the oxygen uptake of one 3-mm. stem (Column 1), two 1½-mm. stems (Column 2) or three 1-mm. stems (Column 3) in the same diver. However, when only one 1-mm. stem was placed in a diver (Column 4), it used nearly half as much as three 1-mm. stems. Similarly, although two 3-mm. stems (Column 5) used the same amount of O_2 as one 6-mm. stem (Column 6), a single 3-mm. stem used $\frac{3}{4}$ as much as two 3-mm. stems or one 6-mm. stem.

Table I also shows that the stems collected early in the summer (from Woods Hole) had appreciably higher O_2 uptake than those collected from Cape Cod Canal, although the general relationships between the effects of size of the piece and crowding appeared to be similar. In order to assess the validity of these impressions the data in each series were calculated as per cent of the O_2 uptake of the

TABLE II

UPTAKE IN PERCENT OF OXYGEN USED BY SINGLE 3MM STEM

Source of Stems	Total Length 3mm 3 mm	1½ mm	1 mm	Total Length 1 mm	Total Length 6mm 3 mm	6 mm
Warm Water (WOODS HOLE)	100%	125%*	91%	36%		
Cold Water (C.COD CANAL)	100%	110%	116%	51%	152%*	152%
MEANS	100%	112%	100%	44%	152%*	152%

* Tentative: More data needed

3-mm. stem which served as "control" for that series, and these are summarized in the next table.

In Table II it is seen that two 1½-mm. stems used 12% more O_2 than one 3-mm., and that three 1-mm. stems used the same amount as the one 3-mm. stem. One 1-mm. stem used 44% of that used by one 3-mm. and two 3-mm. stems or one 6-mm. stem used 152% of that of the 3-mm. control.

Since the data for stems from cold water are more complete than those for stems from warm water, the former were used for a further analysis of the situation. If the cut surface were the determining factor in O_2 uptake, the 1-mm., 3-mm., and 6-mm. pieces should have the same uptake since they all have two cut surfaces; instead, the actual measurements give a 1:2:3 ratio. Likewise, two 1½-mm. pieces should use two times as much O_2 as one 3-mm. piece and three 1-mm. pieces should require three times that of one 1-mm. piece. The actual findings were 10% and 61% increases, respectively.

If O_2 uptake were equal along the entire length of stem, all of the 3-mm. combinations should give the same uptake. The observed findings of 110% for two 1½-mm. pieces and 116% for three 1-mm. pieces could perhaps be reconciled to this hypothesis; however, the observed uptake of the single 1-mm. stem is too high (51% instead of 33% of the 3-mm. uptake) and that of two 3-mm. stems or one 6-mm. stem is too low (152% of the control uptake rather than 200%).

Table III shows the precise relationship between O_2 uptake and length of stem when calculated per cut surface. In the upper row it is seen that each cut surface of a 6-mm. stem was associated with an uptake of oxygen which was 1½ times that of a 3-mm. stem and 2½ times that of a 1-mm. stem. In the second row it is seen that when there were four cut surfaces for 6 mm. of stem (*i.e.*, two 3-mm. pieces)

TABLE III

OXYGEN-UPTAKE PER CUT SURFACE*

No. of Cut Surfaces	Length of Stem 6mm	3mm	1mm
2	8.1	5.6	3.1
4	4.0	3.0	-
6	-	1.9	-

* $mm^3 \times 10^{-2}$ per hour

the uptake per surface was reduced, as was the case with a 3-mm. piece cut into two 1½-mm. pieces. However, oxygen exchange per cut surface again was greater in the longer than the shorter pieces. That this was the result of a true inhibition of oxygen consumption was shown when the uptake per cut surface of two 3-mm. pieces in a diver was compared with that of a single 3-mm. piece (4 as compared with 5.3 $mm.^3 \times 10^{-2}$ per hour) and that of two 1½-mm. pieces or three 1-mm. pieces compared with that of a single 1-mm. piece in a diver (3.0 or 1.9 as compared with 3.1).

The influence of total volume of tissue upon oxygen uptake is shown in Table IV, in which the data on the stems from cold water have been calculated on the basis of uptake of oxygen per millimeter of stem length. These show that when the distance between the two cut surfaces is great the average uptake is small, when the distance is small the average uptake is large. The data are not sufficient to

quantitate the differences in oxygen requirements of the 1–1½ mm. at the two ends of the stem which have been activated by exposure to oxygen and the intervening non-activated stem. However, the difference between the uptake of single 6-mm. stems and single 3-mm. stems (16.1 minus 11.1 $mm.^3 \times 10^{-2}$) suggests that under the conditions of the experiment the non-reconstituting parts of the stem consume oxygen at the rate of something in the order of 2 $mm.^3 \times 10^{-2}$ per millimeter length as compared with 5½ for the ends. Further studies are planned in order to verify this finding.

The lower half of Table IV again shows the inhibitory effects of increasing the number of cut surfaces per mass of tissue when confined in a small volume of

TABLE IV

OXYGEN UPTAKE PER MILLIMETER LENGTH OF STEM*

No. of Cut Surfaces	Length of Stem 6mm	3mm	1mm
2	2.7	3.7	6.2
4	2.7	4.7	-
6	-	4.5	-

* $mm^3 \times 10^{-2}$ per hour

fluid. Because oxygen uptake depends on oxygen concentration of the medium (Barth, 1938), calculations were made to determine the volume of oxygen in the divers at the beginning of the period of measurement. Since the mean volume of the divers was 68.85 $mm.^3$, they contained approximately 13.77 $mm.^3$ of O_2. Even using a rate of O_2 uptake of 30 $mm.^3 \times 10^{-2}$/hr. (higher than any which has been measured), the oxygen in the diver would suffice for 46 hours. Therefore, hypoxia could not have contributed to the reduction in uptake.

Since CO_2 is a potent inhibitor of reconstitution, calculations were made to determine whether the volume of NaOH solution in the necks of the divers was adequate to absorb the CO_2 liberated. Using the same figure of 30 $mm.^3 \times 10^{-2}$/hr.

O_2 uptake the calculations showed that the 2 ml. of NaOH could absorb CO_2 for 75 hours before becoming exhausted. This indicates that some inhibitor other than CO_2 liberated by the cut ends of the stems was responsible for the O_2 depression. Since it has been demonstrated that low pH inhibits reconstitution (Goldin, 1942) and that pH-lowering substances are released during reconstitution (Miller, 1948; Miller and Miller, unpublished data), it is suggested that these substances may depress the O_2 uptake in the divers containing two or more pieces.

Discussion

1. Oxygen uptake and reconstitution

Barth (1940b) was unable to find any differences in O_2 uptake of "regenerating" stems (with open ends) and "non-regenerating" stems (with both ends ligatured) and concluded that very little oxygen was used in regeneration, even though his earlier studies had shown that the process was highly oxygen-dependent. However, there are aspects of his technique that make his findings difficult to interpret. His determinations were made in a Warburg apparatus which was shaken during the measurements. This so greatly increased the oxygen available to the stems that ligatured stems can hardly be considered as resting stems. Indeed, he reported (p. 372) that 50% of one group of ligatured controls formed hydranths. Under ordinary circumstances ligatured stems do not show any visible signs of reconstituting. Therefore, it is possible that the lack of difference in oxygen uptake between ligatured and non-ligatured stems could be attributed to an artificially elevated uptake in the ligatured stems, caused by the shaking in the Warburg apparatus. In spite of this possibility Barth's conclusions regarding oxygen uptake in reconstitution have been widely quoted and have been incorporated in theories of regeneration (Barth, 1940a, 1944; Spiegelman, 1945; Steinberg, 1954, 1955).

In 1953 Sze reported a study of oxygen uptake in *Tubularia* stems using cartesian divers. His technique avoided the problems raised by shaking but encountered other problems which again complicate the interpretation of the results. He found it necessary to make his uptake measurements at a temperature of 25° C. even though the stems had come originally from colder water and had been kept in the laboratory at 15° C. *Tubularia* colonies from colder water that are brought into a laboratory at 25° C. lose their hydranths and may even cytolyze (Moore, 1939). Stem segments are less sensitive than hydranths, but can hardly be considered as normal under such conditions of temperature stress.

In addition, both Barth and Sze reported their measurements in $mm.^3/hr./10$ mg. dry (or in some cases wet) weight. Although theoretically this should be the most precise procedure, in the case of *Tubularia* the presence of the chitinous perisarc introduces a complication which negates its advantages. Since the nonliving perisarc far outweighs the metabolizing tissues of stem, calculations based on weight will contain a large error if there are differences in thickness in different parts of the perisarc. Such differences are slight and probably can be safely disregarded in short pieces from adjacent regions. However, the thickness of the perisarc increases proximad and the differences become appreciable in sections only a few millimeters apart. To avoid this complication, in the present study the lengths and diameters of the stems were measured under magnification and O_2 uptake comparisons were made on the basis of units of stem length.

Our data on the oxygen uptake of pieces less than 3 mm. long do not offer much assistance in resolving the question of whether or not reconstitution is accompanied by an appreciable alteration in oxygen uptake. However, ciliary activity, production of pH-lowering substances and the subsequent differentiation of a hydranth all indicate that under ordinary conditions, from 1 to 1½ mm. of stem subjacent to the cut surface is involved in the activation which follows sectioning. On this basis, the difference in uptake between a 3-mm. and a 6-mm. stem was used to compare the uptake of the peripheral 3 mm. with the interior 3 mm. This showed that the average oxygen uptake per millimeter of stem at the ends of the stem was two times that at the middle (10.6/3 or 3.5, as compared with 5.5/3 or 1.8). We have evidence (unpublished) that there is balance during reconstitution between the level of oxygen available to the cells and the level of pH-lowering substances which accumulate in the stem and which are inhibitory (Goldin, 1942). Because of coelenteric circulation, the concentration of these inhibitors is lower in long than in very short stems (Miller, 1948; Miller and Miller, unpublished data). Therefore, it is entirely possible that when longer stems are measured, the uptake of the reconstituting ends will be found to be appreciably greater than two times that of the resting stem tissue. However, the important fact remains that the measurements reported here bring the changes in oxygen uptake following cutting into a rational relationship to the well known dependence of reconstitution upon oxygen which Barth demonstrated so clearly in 1938. The measurements reported here also accord with studies on the energy requirements in *Corymorpha*, a related species which has a naked stem (Child and Watanabe, 1935), in hydranth development in *Tubularia* embryos (Miller, 1946) and in embryological processes and regenerative phenomena in general (Child, 1941).

2. Oxygen uptake in 1-mm. pieces

Very short pieces present an interesting complication. Since their length is less than that of a normal reconstituting hydranth and they have two surfaces for metabolic exchange, one might expect unusually large hydranth primordia in these short pieces. Such is not the observed result. They often fail to reconstitute at all and when reconstitution does occur, they produce the smallest hydranth primordia or fully formed hydranths that the authors have seen. Other evidence of inhibition in these short pieces is that instead of completing reconstitution in 48 to 60 hours they often require 4 to 5 days.

In spite of this, the single 1-mm. pieces gave the highest per millimeter O_2 uptakes of any of the pieces measured. In a parallel study (Miller and Miller, unpublished data) it has been found that the 1-mm. stems have the lowest average pH of any stems studied. Thus it appears that the antagonism between acid and O_2 reported by Goldin (1942) has a counterpart in reverse within the coenosarc of very short (1-mm.) stems. In spite of increased availability of O_2 for the tissues and increased utilization by them, in the presence of increased acidity reconstitution is delayed, and when it occurs is inhibited (*i.e.*, the scale of organization is reduced). If this picture is a correct one, increasing the O_2 in the sea water should increase the scale of organization (*i.e.*, the size of the organ primordia). When tested, this prediction was verified. Oxygenation so increased the size of the primordia that the pieces were not long enough to produce complete hydranths. As a result there

was a great increase in the number of partial forms possessing a hypostome, distal tentacles and gonophore buds or merely a hypostome and distal tentacles. Measurements showed that 10 times as much tissue was included in the distal tentacles of the latter group as in the distal tentacles of the complete hydranths which developed in the unoxygenated controls (Miller and Miller, unpublished).

3. Inhibition of reconstitution

Reconstitution in *Tubularia* is initiated by the oxygen which enters the stem through the cut surfaces. However, this form is extremely sensitive, and reduction in size or total inhibition of the developing hydranth can be produced by a wide variety of agents of both exogenous and endogenous origin. The present discussion will be limited to naturally occurring inhibitors. In 1939 CO_2 was reported to be a powerful inhibitor (Miller, 1939) and later it was shown that effect was produced by hydrogen ions (Goldin, 1942). Subsequently it was found that pH-lowering substances accumulate in the coelenteron of reconstituting stems and especially in the reconstituting hydranth. The concentration in ligatured stems reaches levels which Goldin found to be inhibitory when externally applied (Miller, 1948; Miller and Miller, unpublished data). At one time Barth (1940) postulated competition for nutritive substances circulating within the coelenteron to explain dominance of the distal over the proximal cut surface. However, his data could be interpreted equally well on the hypothesis that dominance was maintained by a differential susceptibility to inhibitors (Child, 1941). When put to a test, the stems through which fresh filtered sea water flowed throughout the period of regeneration actually produced slightly more hydranths than did controls from which no coelenteric fluid was removed (Miller, 1959). In this experiment any nutritive substances liberated into the coelenteron of the experimental stems were removed before they could reach the distal end, since the flow was from distal to proximal.

Rose and Rose (1941), Rose (1955), Tardent (1955, 1960) and Tweedell (1958) have been interested in inhibitors produced by hydranths and stems. Although Fulton (1959) reported that he obtained inhibition from hydranth water only when he could demonstrate bacterial multiplication in the preparations, he found that preparations either from hydranths or stems made by extraction (Tardent, 1955; Tweedell, 1958) contained inhibitors which were not dependent upon bacterial action (Fulton, 1959, p. 237). Likewise, Rose (1957) reported polarized inhibitory effects in grafting experiments which could not be explained on the basis of contaminants. Also, Beloussov and Geleg (1960) have reported inhibition which was independent of bacterial action.

Although many authors have reported inhibition of regeneration in *Tubularia* resulting from crowding, the present observations are the first which show that under these conditions the oxygen uptake is depressed. Calculations showed that because of the relatively large volume of the air and small volume of sea water in the divers, no oxygen deficiency could develop in the period of the measurements. Likewise, the NaOH in the divers was found to be more than adequate. Thus it was concluded that some other noxious product of metabolism was involved primarily in this effect.

During the first twelve hours of reconstitution the ends of the stems liberate

substances into the coelenteron which increase the hydrogen ion concentration at the ends by a factor of 12 (1.2 pH units) and maintain it at this level throughout the remainder of the reconstitutive period (Miller, 1948; Miller and Miller, unpublished data). This indicates a high rate of production of acidifying substances. As shown by Goldin (1942) a pH of 6.8 in the surrounding sea water will prevent hydranth formation at ordinary levels of oxygenation (5 cc./1.). It is suggested that in the small volume of sea water in the divers, pH may have fallen rather rapidly to inhibitory levels.

It must be emphasized, however, that these studies were made during the first 6–8 hours after cutting. They give information only during the migratory phase of reconstitution. Other and organ-specific inhibitors, such as those indicated in Rose's work (1957), undoubtedly operate during later stages. They may likewise affect oxygen uptake but as yet there is no information on this question.

Summary

Oxygen uptake measurements were made in cartesian divers on 43 pieces of *Tubularia* stems between 1 mm. and 6 mm. in length with the following findings:

1. The 1-mm. stems had the highest, 3-mm. stems the next highest and 6-mm. stems had the lowest uptake when calculated per millimeter of length of stem.

2. By comparing uptake of 3-mm. and 6-mm. stems it was found that the middle 3 mm. of the 6-mm. stems used O_2 at less than half the rate of the two ends. This is in disagreement with the conclusions of Barth and Sze that regeneration does not involve any appreciable increase in oxygen requirements.

3. When two or more pieces were placed in the same diver their oxygen uptakes were depressed. Calculations showed that neither hypoxia nor hypercapnia could have caused this depression. It was suggested from other studies that acid metabolites liberated through the cut surface may have caused the observed effects.

LITERATURE CITED

Barth, L. G., 1938. Quantitative studies of the factors governing rate of regeneration in *Tubularia*. *Biol. Bull.*, **74**: 155–177.

Barth, L. G., 1940a. The process of regeneration in hydroids. *Biol. Rev.*, **15**: 405–420.

Barth, L. G., 1940b. The relation between oxygen consumption and rate of regeneration. *Biol. Bull.*, **78**: 366–374.

Barth, L. G., 1944. The determination of the regenerating hydranth in *Tubularia*. *Physiol. Zoöl.*, **17**: 355–366.

Beloussov, L., and S. Geleg, 1960. Chemical regulation of the morphogenesis of hydroid polyps. *Doklady-Acad. of Sci., SSSR*, **130**: 1165–1168.

Bickford, E. E., 1894. Notes on regeneration and heteromorphosis of tubularian hydroids. *J. Morphol.*, **9**: 417–430.

Child, C. M., 1941. Patterns and Problems of Development. University of Chicago Press, Chicago, Illinois.

Child, C. M., and Y. Watanabe, 1935. Differential reduction of methylene blue by *Corymorpha palma*. *Physiol. Zoöl.*, **8**: 395.

Claff, C. L., 1947. "Braking" pipettes. *Science*, **105**: 103–104.

Fulton, C., 1959. Re-examination of an inhibitor of regeneration in *Tubularia*. *Biol. Bull.*, **116**: 232–238.

Goldin, A., 1942. A quantitative study of the interrelationship of oxygen and hydrogen ion concentration in influencing *Tubularia* regeneration. *Biol. Bull.*, **82**: 340–346.

Holter, H., 1943. Technique of the Cartesian diver. *C. R. Lab. Carlsberg*, **24**: 399–478.

Hyman, L. H., 1926. The axial gradients in Hydrozoa. VIII. Respiratory differences along the axis in *Tubularia* with some remarks on regeneration rate. *Biol. Bull.*, **50**: 406–426.

Linderstrøm-Lang, K., 1943. On the theory of the Cartesian diver micro respirometer. *C. R. Lab. Carlsberg*, **24**: 333–398.

Miller, J. A., Jr., 1937. Some effects of oxygen on polarity in *Tubularia crocea*. *Biol. Bull.*, **73**: 369.

Miller, J. A., Jr., 1939. Experiments on polarity determination in *Tubularia* regenerates. *Anat. Rec.*, **75**: 38–39.

Miller, J. A., Jr., 1940. Oxygenation and ciliary rate in regenerating *Tubularia* stems. *Bull. Mt. Desert Island Biol. Lab.*, 41–44.

Miller, J. A., Jr., 1942. Some effects of covering the perisarc upon tubularian regeneration. *Biol. Bull.*, **83**: 416–427.

Miller, J. A., Jr., 1946. Differential reduction of Janus green in the early development of *Tubularia crocea*. *Anat. Rec.*, **94**: 17.

Miller, J. A., Jr., 1948. pH estimations in reconstituting pieces of tubularian stems. *Biol. Bull.*, **95**: 243.

Miller, J. A., Jr., 1959. Nutritive substances and reconstitution in *Tubularia*. *Proc. Soc. Exp. Biol. Med.*, **100**: 186–189.

Miller, J. A., Jr., L. L. Philpott and C. L. Claff, 1961. Oxygen uptake in short pieces of *Tubularia* stems. *Biol. Bull.*, **121**: 398.

Moore, J. A., 1939. The role of temperature in hydranth formation in *Tubularia*. *Biol. Bull.*, **76**: 104–107.

Rose, S. M., 1955. Specific inhibition during differentiation. *Ann. N. Y. Acad. Sci.*, **60**: 1136–1159.

Rose, S. M., 1957. Polarized inhibitory effects during regeneration in *Tubularia*. *J. Morphol.*, **100**: 187–205.

Rose, S. M., and F. C. Rose, 1941. The role of the cut surface in *Tubularia* regeneration. *Physiol. Zoöl.*, **14**: 328–343.

Spiegelman, S., 1945. Physiological competition as a regulatory mechanism in morphogenesis. *Quart. Rev. Biol.*, **20**: 121–146.

Steinberg, M. S., 1954. Studies on the mechanism of physiological dominance in *Tubularia*. *J. Exp. Zool.*, **127**: 1–26.

Steinberg, M. S., 1955. Cell movement, rate of regeneration, and the axial gradient in *Tubularia*. *Biol. Bull.*, **108**: 219–234.

Sze, L. C., 1953. Respiratory gradients in *Tubularia*. *Biol. Bull.*, **104**: 109–113.

Tardent, P., 1955. Zum Nachweis eines regenerationshemmenden Stoffes in Hydranth von *Tubularia*. *Rev. Suisse Zool.*, **62**: 289–294.

Tardent, P., 1960. Principles governing the process of regeneration in hydroids. *Symp. Study Devel. Growth*, **18**: 21–43.

Tweedell, K. S., 1958. Inhibitors of regeneration in *Tubularia*. *Biol. Bull.*, **114**: 255–269.

Zwilling, E., 1939. The effect of removal of perisarc on regeneration in *Tubularia crocea*. *Biol. Bull.*, **76**: 90–103.